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Original Research

Different strains of *Acinetobacter baumannii* spreading in an intensive care unit

Chien-Hsien Huang [a,b,d], Chia-Lin Lee [b], Aming Chor-Ming Lin [c,d], Wei-Yu Chen [a,b,d], Pi-Chu Teng [b], Shu-Hua Lee [b], Yi-Jan Hsieh [b], Tsrang-Neng Jang [a,b,d,*]

[a] *Division of Infectious Diseases, Department of Internal Medicine, Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan*
[b] *Division of Infection Control, Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan*
[c] *Emergency Department, Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan*
[d] *Department of Internal Medicine, School of Medicine, Fu-Jen Catholic University, Taiwan*

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Abstract

Background: An outbreak of *Acinetobacter baumannii* in neurological intensive care unit was detected, causing nosocomial infection on 6 cases and colonization on 5 cases.
Methods: Five major clones of *A baumannii* circulating in the intensive care unit were detected by pulse-field gel electrophoresis (PFGE). The outbreak was controlled by aggressive intervention with disinfection of equipments and environment, and audit of hand-washing practice by the staff in this intensive care unit.
Results: The *A baumannii* could be isolated from equipments and the environment, including the chart folders 5/8 (62.5%), work trolley 1/2 (50.0%), ambulance bag of patient 1/3 (33.3%), hands of healthcare-workers 7/22 (31.8%), surfaces of monitors 1/4 (25.0%), bottle of suction fluid 1/4 (25.0%); but not isolated from the hands of healthcare-workers after disinfection with wash practice.
Conclusions: Different strains of *A baumannii* may cause infection, exist in medical environment, and colonize the patients at the same time. It may develop into true infection anytime. We conclude that hand hygiene as well as disinfection of equipments and the environment are the two most important factors to control and prevent the outbreak of *A baumannii*.

Keywords: *Acinetobacter baumannii*; Nosocomial infection; Intensive care unit

1. Introduction

Acinetobacter baumannii is a Gram-negative coccobacillus that is found in many hospital environments and can be isolated from the skin and feces of healthy populations.[1] *A baumannii* can proliferate in wet conditions and survive in a poor environment for as long as 13 days on the surface of a completely dry substance.[2] It is a low-virulence organism that has rarely caused invasive diseases in the past, but has emerged as a clinically important nosocomial pathogen in recent years, especially in critically ill patients.[3–5] Underlying conditions associated with *A baumannii* bacteremia include alcoholism, burns, malignancy, intracranial hemorrhage, and trauma.[6,7] *A baumannii* causes nosocomial infection in patients with pneumonia, septicemia, endocarditis, meningitis, brain abscess, lung abscess and urinary tract infection.[3,8]

In nosocomial infection and outbreaks, the major transmission route of *A baumannii* is contact transmission, either directly from the hands of healthcare workers or indirectly via environmental colonization.[9] Most outbreaks are caused by contamination from mechanical ventilators, central venous systems and invasive procedures.[10–12] *A baumannii* has been isolated from gloves, water, intravenous fluid, monitors and

* Corresponding author. Division of Infectious Diseases, Department of Internal Medicine, Shin Kong Wu Ho-Su Memorial Hospital, 95 Wen Chang Road, Shih Lin District, Taipei 111, Taiwan.
E-mail address: m002137@ms.skh.org.tw (T.-N. Jang).

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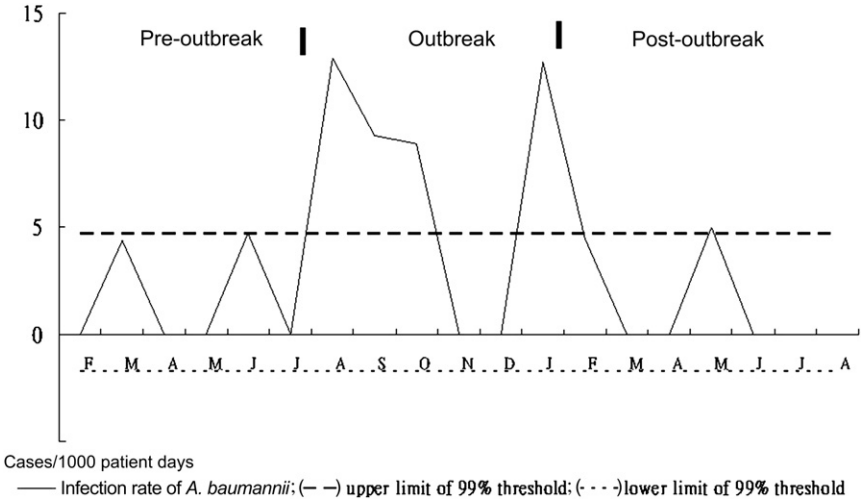

Fig. 1. Monthly infection rate of *A baumannii* from February 2002 to August 2003.

beds. Major risk factors for outbreaks include burn injury, major operations, the use of steroids and invasive procedures.[3,8] Most *A baumannii* infections are acquired in hospital and are directly proportional to the length of hospital stay, especially in the intensive care unit.[9] The most common primary site for *A baumannii* bacteremia is the respiratory tract.[13,14] The presentation of this organism can range from colonization and self-limiting infection to serious life-threatening pneumonia and septicemia, especially in critically ill patients.[13–16]

An *A baumannii* outbreak causing pneumonia, urinary tract infection, soft tissue infection, catheter-related infection and bacteremia was detected by our routine surveillance system in the neurological intensive care unit (NICU) in Shin Kong Wu Ho-Su Memorial Hospital, Taiwan. In this study, we used molecular methods to investigate this event and implemented strict infection control measures to control the outbreak.

2. Materials and methods

2.1. Background

An infection control and surveillance system was established in 1992 in Shin Kong Memorial Hospital, a 961 bed medical center in Taipei, Taiwan. The definition of nosocomial infection was guided by the National Nosocomial Infections Surveillance System.[17] Increasing numbers of infectious cases of *A baumannii* were detected by our routine surveillance system in NICU in the 7 months from August 2002 to February 2003 (Fig. 1). During the outbreak, 11 cases of *A baumannii* nosocomial infection were found. The endemic rate for *A baumannii* as a direct cause of hospital-acquired infection in our NICU before this outbreak was 1.52/1000 patient-days, whereas during the outbreak it was 6.94/1000 patient-days ($p = 0.045$). The endemic rate returned to 0.76/1000 patient-days after the outbreak (Table 1). During the outbreak, contact precautions were applied to all patients with infection or colonization of *A baumannii.* A microbiological survey of patients, equipment, healthcare workers' hands and the environment was done during the outbreak and any episode of infection caused by *A baumannii* was included. The surveillance of patients continued until discharge from NICU or no further clinical infection was detected. The incidence of *A baumannii* infection returned to normal after these 7 months (Fig. 1).

2.2. Bacterial isolates

Sampling of specimens taken from patients, equipment, the environment and the healthcare workers' hands was done during the outbreak. A total of 46 cultures were obtained from the environment, including resuscitation bags, suction fluid bottles, surfaces of respirator monitors, aerosolizing water for ventilators, the blades of intubation scopes, the surfaces of monitors, computer keyboards, chart folders, work trolleys, telephones, and cleaning wipes. The healthcare workers' hands were sampled twice at an interval of 2 weeks. Cultures were obtained from doctors, nurses, respiratory therapists, assistants, and cleaning staff by washing their hands with 50 mL of normal saline.

Table 1
The infectious rate of patients in pre-outbreak, outbreak and post-outbreak

	Nosocomial *A baumannii* infection case	Total admission cases	Admission days	Incidence/1000 patient-days (‰)
Pre-outbreak	2	137	1320	1.52
Outbreak	11	155	1584	6.94[a]
Post-outbreak	1	138	1315	0.76[b]

[a] Compare between pre-outbreak and outbreak; $p = 0.045$.
[b] Compare between outbreak and post-outbreak; $p = 0.027$.

2.3. Antimicrobial susceptibility

All isolates obtained for surveillance purposes were identified by the API 20 NE system. (API-BioMérieux, LaBalme Les Grotles, France) Antibiotic susceptibility was tested in the clinical microbiology laboratory by the standard disc diffusion method, according to the recommendations of the Clinical and Laboratory Standards Institute.[18] Susceptibility tests were done for gentamicin, amikacin, isepamicin, minocycline, trimethoprim-sulfamethoxazole, levofloxacin, ciprofloxacin, ticarcillin/clavulanate, piperacillin, pipracillin/tazobactam, aztreonam, ceftazidime, cefepime, imipenem/cilastatin and meropenem.

2.4. Pulse-field gel electrophoresis

Pulse-field gel electrophoresis (PFGE) was used to examine the 26 available preserved isolates of *A baumannii* (some cultures had no bacterial growth). The pure bacterial cultures were embedded into plugs, placed into EC buffer (6 mM Tris, 1 M NaCl, 0.1 M EDTA, 0.2% sodium deoxycholate, 0.5% sarkosyl, pH 8) for 4−5 hours, then incubated with 50 μL proteinase K (20 mg/dL) overnight at 50°C. DNA was extracted and purified were done by standard procedures, and digested by the restriction endonuclease SmaI. The restriction fragments were separated by PFGE in a CHEF unit (CHEF Mapper XA System, Bio-Rad Laboratories) using 0.5 × TBE buffer (Bio-Rad Laboratories, Beverly, MA, USA) at 12°C, 6 V/cm, with a switch time of 5−20 seconds for 22.5 hours and a Lambda Ladder PFG Marker (Biolab Laboratories, Beverly, MA, USA) as molecular mass marker. Interpretation of the PFGE types followed the description by Tenover et al[19] Similar PFGE patterns of the isolates were considered to be closely related isolates if the number of fragment differences was $\leq$3.

2.5. Statistical analysis

Routine surveillance of nosocomial infection was done in each intensive care unit in our hospital to detect abnormally increased numbers and rates of infections compared to the infection rate over the previous 6 months. Any increased infection rate compared to the average rate for 6 months was discussed by the infection control committee and an outbreak was defined as an infectious rate significantly ($p < 0.05$) above the endemic rate. Data were assessed by independent samples t tests (SPSS Inc, Chicago, IL, USA).

3. Results

3.1. Epidemiology investigation of the outbreak

Before the outbreak, there had been only two cases of pneumonia and eight cases of colonization of *A baumannii* in the previous 6 months. However, during the 7-month outbreak, there were seven cases of pneumonia, one case of urinary tract infection, one case of soft tissue infection, one case of catheter-related infection, and one case of bacteremia. The infection rates for the 6 months prior to the outbreak, the outbreak and 6 months after the outbreak were compared. The *p* values were 0.045 for outbreak *versus* pre-outbreak and 0.027 for outbreak *versus* post-outbreak (Table 1). During the outbreak, samples from all patients with infection in the intensive care unit until discharge or 2 months after transfer were cultured.

3.2. Containment measures

Intervention in this intensive care unit was started after the increased infection rate was reported. The microbiological surveillance revealed *A baumannii* colonized on environmental surfaces and healthcare workers' hands. Thereafter, infection control methods, including strict daily environmental cleaning with 0.05% NaOCl, effective sterilization of reusable medical equipment, attention to proper hand hygiene, cohorting and isolation, were implemented by our infection control nurses.

3.3. Bacterial analysis

Cultures of 46 samples from the environment and 44 samples from the healthcare workers' hands were done twice during the outbreak. Thirty isolates of *A baumannii* were preserved, ten from patients and 20 from surveillance cultures. Of the preserved isolates, 26 could be retrieved for PFGE (some failed to grow), including seven from patients, 12 from the environment and seven from the healthcare workers' hands. In addition to *A baumannii*, Enterobacteriaceae, staphylococcus, streptococcus and glucose non-fermenting Gram-negative bacilli were isolated from the surveillance cultures. *A baumannii* was isolated from 62.5% (5/8) of chart folders, 50.0% (1/2) of work trolleys, 33.3% (1/3) of resuscitation bags, 31.8% (7/22) of healthcare workers' hands before handwashing practice, 25.0% (1/4) of the surfaces of respiratory monitors, 25.0% of suction meters (1/4), 25.0% of suction fluid bottles (1/4), 25.0% of aerosolizing water for ventilators (1/4), and 25.0% of the surfaces of central monitors (2/8). No *A baumannii* was isolated from healthcare workers' hands after handwashing practice was begun (Fig. 2).

PFGE showed five outbreak strains, types A−E, and another six epidemiologically unrelated strains, types F−K, were detected only in the environmental surveillance (Table 2). Case 1: the first case of strain type A was in a patient with a cerebral vascular accident who was transferred from the ordinary ward because of respiratory failure. Positive sputum cultures of *A baumannii* were found 1 month before the outbreak and in pus/wound during the outbreak. Case 2: Strain type A was isolated from the sputum 1 week later. Case 3: Strain type B was isolated from the sputum. Case 4: Strain type B was isolated from a work trolley and the hands of nurse A. Strain type C was isolated from the sputum of Case 4, the aerosolizing water of Case 4, nurse B, and the chart of Case 6. Strain type D was isolated from the urine of Case 5 and the sputum of Case 6 (10 days after Case 5), the

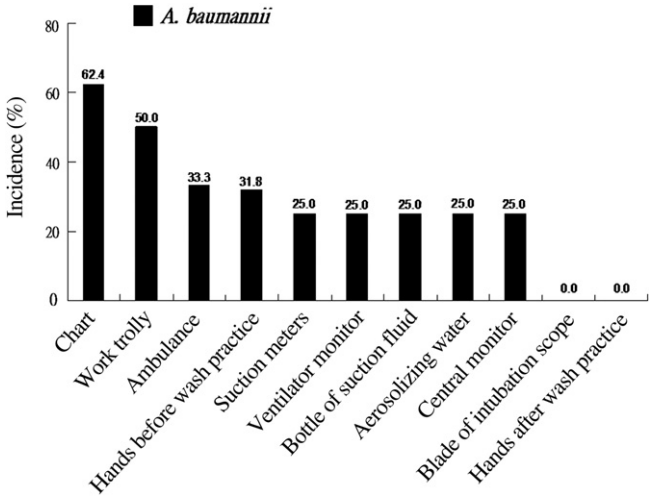

Fig. 2. *A baumannii* colonization in the environment and on healthcare workers' hands.

ambulance of Case 6, the central monitors of Cases 8 and 9, and the hands of nurse C. Strain type E was isolated from the central monitor and suction bottle of Case 3, the sputum of Case 7 and the hands of nurse E.

PFGE showed five outbreak types of *A baumannii* in patients and the environment. Drug susceptibility was analyzed in all of the isolates, and several different patterns were found (Table 2).

4. Discussion

The infection and transmission of emerging pathogens, including *A baumannii,* have increased in most hospitals in recent years.[3,5,7,9] The frequency of nosocomial infections with *A baumannii* is increasing; clone transmission and outbreaks are emerging and there are ongoing problems in many hospitals, especially in intensive care units.[5,12] Colonization can lead to clinical infections, including bacteremia, pneumonia, urinary tract infections, soft tissue infections and surgical site infections.

Outbreaks result in prolonged hospitalization and increased costs, so it is important to investigate and stop outbreaks quickly to prevent increased morbidity, mortality and costs. Interventions include emphasis on infection control, promotion of hand hygiene, daily disinfection of all equipment and surfaces of monitors, use of disposable saline and drug bottles and aggressive cleansing of all surfaces in contact with patients and healthcare workers. These interventions were monitored by infection control nurses in this intensive care unit for several months.

The colonization rate on hands decreased dramatically after strict handwashing practice. Colonization of any bacteria on hands was 91% (20/22) before handwashing practice and decreased to 13.6% (3/22) afterward. In addition, *A baumannii* was isolated from 7/22 hands before handwashing practice and none after; handwashing decreased the rate of colonization *of A baumannii* and other bacteria (Fig. 2). Enterobacteriaceae

Table 2
Drug susceptibility and pulse-field gel electrophoresis (PFGE) types of the isolated *A baumannii*

Strain	Source of isolates	Specimen	PFGE type	GM	PIP	CAZ	ATM	TZP	CIP	IPM	FEP	AN
1	Case 1	Pus	A	S	R	R	I	I	R	S	I	S
2	Case 2	Sputum	A	R	R	R	I	I	R	S	I	R
3	Case 3	Sputum	B	S	I	S	I	S	S	S	S	S
4	Nurse A	Hands	B	S	S	S	I	S	S	S	S	S
5	Case 4	Trolley	B	S	S	S	I	S	S	S	S	S
6	Case 4	Sputum	C	R	S	S	I	S	S	S	S	R
7	Nurse B	Hands	C	R	S	S	R	S	S	S	S	R
8	Case 4	Aerosolizing water	C	R	S	S	I	S	S	S	S	R
9	Case 6	Chart	C	R	S	S	I	S	S	S	S	R
10	Case 8	Chart	C1	R	S	I	I	S	I	S	R	I
11	Case 5	Urine	D	R	R	R	R	R	R	S	R	R
12	Case 9	Central monitor	D	R	R	R	R	R	R	S	R	R
13	Case 6	Ambulance	D	R	R	R	R	R	R	S	R	R
14	Case 8	Central monitor	D	R	R	R	R	R	R	S	R	R
15	Case 6	Sputum	D	R	R	R	I	S	R	S	I	R
16	Nurse C	Hands	D	R	R	R	I	R	R	S	R	R
17	Nurse E	Hands	E	R	I	S	R	S	S	S	S	R
18	Case 3	Central monitor	E	R	I	S	R	S	S	S	S	R
19	Case 3	Suction bottle	E	R	I	S	R	S	S	S	S	R
20	Case 7	Sputum	E	R	I	S	R	S	S	S	S	R
21	Nurse D	Hands	F	S	I	S	I	S	S	S	S	S
22	Case 3	Suction monitor	G	R	I	S	R	S	S	S	S	R
23	Case 10	Chart	H	S	S	S	I	S	S	S	S	S
24	Nurse F	Hands	I	S	S	S	I	S	S	S	S	S
25	Case 9	Chart	J	S	S	S	I	S	S	S	S	S
26	Nurse G	Hands	K	S	I	S	I	S	S	S	S	S

AN = amikacin; ATM = aztreonam; CAZ = ceftazidime; CIP = ciprofloxacin; FEP = cefepime; GM = gentamicin; I = intermediate; IPM = imipenem; PIP = piperacillin; R = resistant; S = sensitive; TZP = piperacillin/tazobactam.

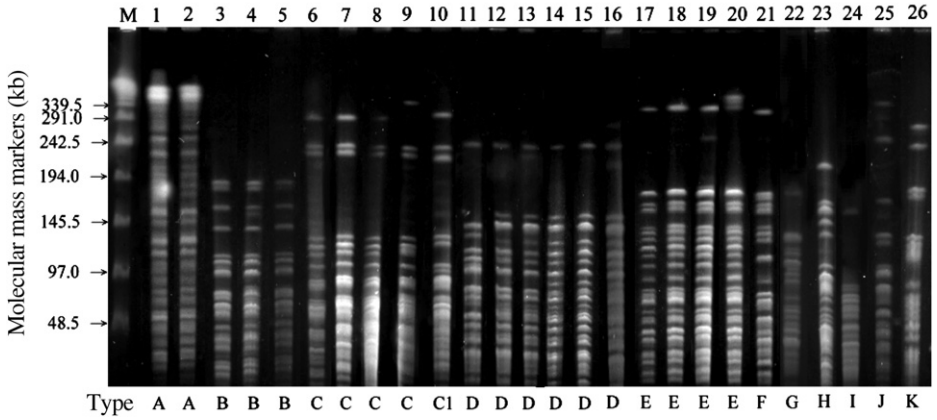

Fig. 3. Pulse-field gel electrophoresis patterns of the *A baumannii* isolates. 1:Case 1, pus 10:Case 8, chart 19:Case 3, suction bottle. 2:Case 2, sputum 11:Case 5, urine 20:Case 7, sputum. 3:Case 3, sputum 12:Case 9, central monitor 21:Nurse D. 4:Nurse A 13:Case 6, ambulance 22:Case 3, suction monitor. 5:Case 4 work trolley 14:Case 8, central monitor 23:Case 10 chart. 6:Case 4, sputum 15:Case 6, sputum 24:Nurse F. 7:Nurse B 16:Nurse C 25:Case 9, chart. 8:Case 4, aerosolizing water 17:Nurse E 26:Nurse G. 9:Case 6, chart 18:Case 3, central monitor.

from environmental colonization was isolated mostly from water-containing equipment and hands. Gram-positive bacilli and enterococci were isolated from dry surfaces of various monitors. Nevertheless, *A baumannii* could be isolated from any available specimen; and could be preserved on moist and dry surfaces of equipment, water and hands. Therefore, decontamination and disinfection of the environment and equipment were used to contain this outbreak and to prevent another outbreak in this unit.

A total of 11 different PFGE types of *A baumannii* were detected in the patients and environmental surveillance during this outbreak, but only five types (A−E) that were isolated from our patients were responsible for this outbreak (Fig. 3). Strain type A might have been transmitted from Case 1 to Case 2, resulting in soft tissue infection in Case 1, and pneumonia 1 week later in Case 2. Strain type B caused a lower respiratory tract infection in Case 3 and was colonized on the hands of nurse A and a work trolley in Case 4. Strain type C might have been colonized in the aerosolizing water of Case 4, resulting in pneumonia. Strain type C was detected also on the hands of nurse B and on the charts of Cases 6 and 8. Strain type D caused a urinary tract infection in Case 5 and pneumonia 10 days later in Case 6. Strain type D was also found colonized on the ambulance of Case 6, the central monitors of Cases 8 and 9 and the hands of nurse C. Strain type E caused pneumonia in Case 7 and colonized on the central monitor and suction bottle of Case 3. Different strains could colonize on equipment and in the environment and be transmitted by healthcare workers' hands. We found epidemiologically unrelated strains (types F−K) in the environment. Although they were not pathogens in this outbreak, they could have caused another outbreak or infection later. Routine disinfection of the environment and continuous education of all staff (nurses, doctors, respiratory care workers and cleaners) are important. Different isolates had different drug sensitivities and different PFGE types. They are impossible to differentiate on the basis of susceptibility alone. Molecular methods have recently become the gold standard for typing in outbreak investigations. The sources of outbreaks might be overlooked or misidentified without molecular evidence.

5. Conclusions

An outbreak is big challenge for hospitals and doctors and it might be the first sign of an emerging infection or development of new drug resistance. Evidence of microorganisms was used to verify the existence of this outbreak, which was interrupted by disinfection, handwashing practice and other interventions provided by infection control nurses. The disinfection of equipment, environment cleansing, and persistent promotion of hand hygiene are important in the control and prevention of outbreaks. Surveillance of nosocomial infections should be continuous in any hospital and offers opportunities to predict and prevent outbreaks. Emergency departments and intensive care units are both special care units. Doctors and healthcare workers might be too busy to adhere to proper hand hygiene practices. In addition, emergency departments tend to be crowded, which provides a good opportunity for *A baumannii* to colonize and circulate. Strict contact precautions and vigorous environmental cleansing are mandatory to prevent transmission of this pathogen.

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References

1. Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. *Clin Microbiol Rev.* 2008;21:538−582.
2. Wendt C, Dietze B, Dietz E, Ruden H. Survival of *Acinetobacter baumannii* on dry surfaces. *J Clin Microbiol.* 1997;35:1394−1397.
3. Gales AC, Jones RN, Foreard KR, Linares J, Sader HS, Verhoef J. Emerging importance of multidrug-resistant Acinetobacter species and *Stenotrophomonas maltophilia* as pathogens in seriously ill patients: geographic

patterns, epidemiological features, and trends in the SENTRY Antimicrobial Surveillance program (1997−1999). *Clin Infect Dis*. 2001;32:104−113.
4. Urban C, Segal-Maurer S, Rahal JJ. Considerations in control and treatment of nosocomial infections due to mutidrug-resistant *Acinetobacter baumannii*. *Clin Infect Dis*. 2003;36:1268−1274.
5. Hsueh PR, Teng LJ, Chen CY, et al. Pan drug-resistant *Acinetobacter baumannii* causing nosocomial infections in a university hospital, Taiwan. *Emerg Infect Dis*. 2002;8:827−832.
6. Koprnova J, Svetlansky I, Babel'a R, et al. Prospective study of antibacterial susceptibility, risk factors and outcome of 157 episodes of *Acinetobacter baumannii* bacteremia in 1999 in Slovakia. *Scand J Infect Dis*. 2001;33:891−895.
7. Cisneros JM, Rodriguez-Bano J. Nosocomial bacteremia due to *Acinetobacter baumannii*: epidemiology, clinical features and treatment. *Clin Microbiol Infect*. 2002;8:687−693.
8. Mahgoub S, Ahmed J, Glatt AE. Underlying characteristics of patients harboring highly resistant *Acinetobacter baumannii*. *Am J Infect Control*. 2002;30:386−390.
9. Villers D, Espaze E, Coste-Burel M, et al. Nosocomial *Acinetobacter baumannii* infections: microbiological and clinical epidemiology. *Ann Intern Med*. 1998;129:182−189.
10. Husni RN, Goldstein LS, Arroliga AC, et al. Risk factors for an outbreak of multi-drug-resistant Acinetobacter nosocomial pneumonia among intubated patients. *Chest*. 1999;115:1378−1382.
11. Mulin B, Rouget C, Clement C, et al. Association of private isolation rooms with ventilator-associated *Acinetobacter baumannii* pneumonia in a surgical intensive-care unit. *Infect Control Hosp Epidemiol*. 1997;18:499−503.
12. Theaker C, Azadian B, Soni N. The impact of *Acinetobacter baumannii* in the intensive care unit. *Anaesthesia*. 2003;58:271−274.
13. Jang TN, Kuo BI, Shen SH, et al. Nosocomial Gram-negative bacteremia in critically ill patients: epidemiologic characteristics and prognostic factors in 147 episodes. *J Formos Med Assoc*. 1999;98:465−473.
14. Fagon JY, Chastre J, Domart Y, Trouillet Gibert C. Mortality due to ventilator-associated pneumonia or colonization with Pseudomonas or Acinetobacter species: assessment by quantitative culture of sample obtained by a protected specimen brush. *Clin Infect Dis*. 1996;23:538−542.
15. Porzecanski I, Bowton D. Diagnosis and treatment of ventilator-associated pneumonia. *Chest*. 2006;130:597−604.
16. Kollef MH, Morrow LE, Niederman MS, et al. Clinical characteristics and treatment patterns among patients with ventilator-associated pneumonia. *Chest*. 2006;129:1210−1218.
17. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control*. 2008;36:309−332.
18. Performance Standards for Antimicrobial Susceptibility Testing. *16th Informational supplement (disk diffusion supplemental tables). CLSI document M100-S16 (M2), supplement to CLSI document M2-A9 (disk diffusion)*; 2006.
19. Tenover FC, Arbeit R, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulse-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol*. 1995;33:2233−2239.